Intra-arterial Injections of Histamine, Serotonin, or Bradykinin: A Topographic Study of Vascular Leakage[1] (35072)

G. GABBIANI, M. C. BADONNEL, AND G. MAJNO

Département de Pathologie, Université de Genève, 40 bd. de la Cluse, 1205 Geneva, Switzerland

Ever since it has been possible to identify blood vessels that are abnormally permeable by the technique of vascular labeling (1, 2), it has been noticed that the venules are especially prone to become leaky; in particular, they are selectively affected by those substances that induce vascular leakage and are known as histamine-type mediators (1, 5, 6).

The reason for this special behavior of the venules is not known. Since it was recently shown that leakage occurs through gaps developing as a result of endothelial contraction (7), it is possible that the endothelium of the venules is intrinsically different from that of other small vessels. On the other hand, it is also possible that the special sensitivity of the venules is merely apparent, and somehow related to the fact that the mediator (in all the models studied so far) reaches the vessels from the tissue spaces: whether the mediator be exogenous, after local injection (2, 5, 6) or endogenous, after local or systemic administration of histamine-releasing substances (2–4). It is conceivable that if the mediator reached the microcirculation from the endothelial side—*i.e.*, from the side of the probable target cells—the topagraphic distribution of the leaking vessels may be different.

The primary purpose of the experiments here reported was to test this possibility. Histamine, serotonin or bradykinin were injected either into the common carotid or into the internal spermatic artery of the rat, and the leaking vessels were labeled with carbon black. Since both arteries supply organs of widely different structure and function, it was also possible to compare the relative sensitivity of the vessels in these organs with regard to the histamine-type response.

Materials and Methods. 110 male Wistar rats from the Ivanovas farm (Kissleg-Allgäu, Germany) weighing 120–280 g were divided into 11 equal groups for two series of experiments. In the first series (70 rats, intra-arterial injection of mediator) 10 rats were kept as untreated controls; the remaining rats were injected, in groups of 10, according to the following scheme: under ether anesthesia each rat received first an intravenous injection of a 20% solution of carbon black in NaCl 0.9% (Pelikan "biological ink" batch No. C11/1431a, John Henschel and Co., Farmingdale, N.Y.) and immediately thereafter one of the intra-arterial injections described below (we injected the colloidal marker first so that it would be present and well distributed in the vascular tree when the latter would be reached by the mediator). Intra-arterial injections: A) *in the right common carotid artery* (Table I): 1 ml of NaCl 0.9% either alone or with histamine phosphate (Laboratories Vifor, Geneva, Switzerland, 150 μg/100 g of body weight), 5-HT (serotonin creatinine sulfate, Merck Co., Darmstadt, Germany, 15 μg/100 g body weight) or bradykinin (bradykinin acetate, Sandoz Co., Basle, Switzerland, 100 μg/100 g body weight); B) *in the left internal spermatic artery* (Table II): 1 ml of NaCl 0.9% either alone or with histamine (150 μg/100 g body weight). For the injections in the spermatic artery, we used animals above 230 g and a 32-gauge needle (9). In both arteries, the fluid was injected very slowly, over a period of 1–2 min. To help hemostasis (particularly in the carotid artery) a small square of Gelfoam (Upjohn Co., Kalamazoo, Mich-

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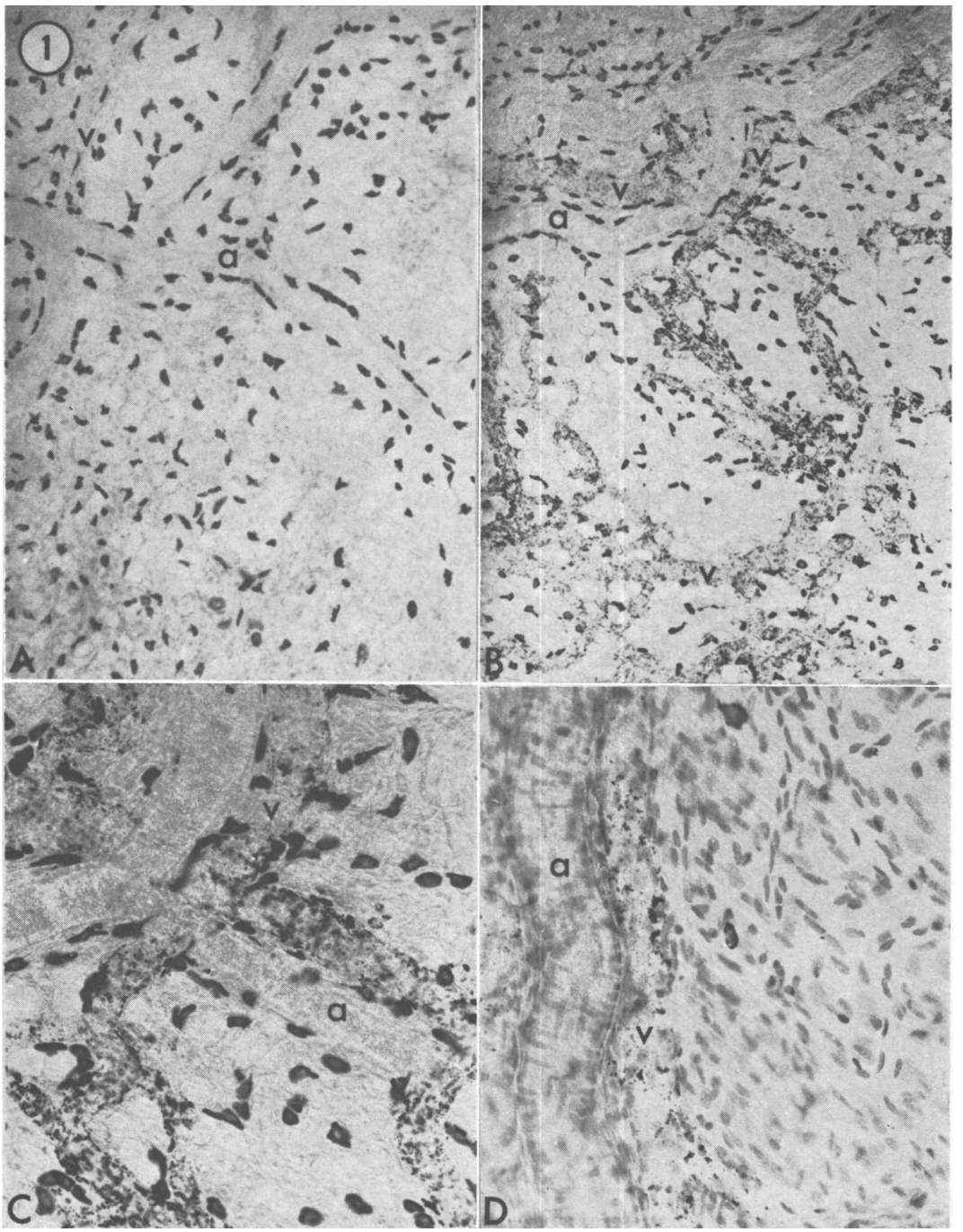

FIG. 1. Carbon deposition after intracarotid injections of mediators. A. A rat treated with NaCl: no labeling is visible in the periosteum of the calvarium (×100, a = arteriole, v = venule). B. A rat treated with 5-HT: intense labeling of several vessels; many mast cells are visible (×100). C. At higher magnification the labeled vessels can be recognized as venules (×250). D. Venular labeling in the dura mater after histamine; note the presence of only a few mast cells in the connective tissue of this membrane (×250).

TABLE I. Vascular Labeling After Injection of a Chemical Mediator in the Common Carotid Artery.

Gr	Treatmnt	Vascular labeling								
		Calvarium	Dura mater	Skin	Lip	Anterior hypophysis	Brain	Cerebellum	Gasserian ganglion
1	NaCl	0	0	0	0	0	0	0	0
2	Histamine	+	+ +	+ +	+ +	+ +	0	0	0
3	5-HT	+	+ +	+ +	+ +	+ +	0	0	0
4	Bradykinin	+	+ +	+ +	+ +	+ +	0	0	0

igan) was applied at the site of injection.

The *second series* (40 rats, intratesticular injection of histamine) was added when it became apparent that intra-arterial mediators did not cause labeling of the testicular vessels. All 40 rats received first (under ether anesthesia) an intravenous injection of carbon black as above; immediately thereafter the abdomen was opened, the left testis was exposed and injected with 0.1 ml of 0.9% NaCl, either alone (10 rats or with histamine, 2.5 μg/100 g (10 rats). It was theoretically possible that the testicular vessels failed to develop labeling not because they were unaffected by the mediator, but because the testicular capsule, the albuginea, was tight enough to oppose the exudation of fluid. Thus, the last set of experiments just described was repeated in 20 rats in which the albuginea had been slit over a length of 5 mm immediately before the local injection. Care was taken to inject the mediator deeply into the testicular parenchyma, and as far as possible from the incision.

Since it was expected that these 40 animals might not show any vascular labeling in the testis, they were all given two control injections subcutaneously (NaCl; histamine, same dosage as above) to prove that they were able to develop a histamine-type response in other sites.

All animals were killed with chloroform 24 hr after the experiment, dissected, and inspected grossly. To better appraise the topography of the labeled vessels, three membranes of tissue were dissected out and mounted on glass slides *in toto*: the periosteum of the calvarium, the dura mater, and the small mesothelial membrane connecting the epididymis and the testis. These tissue membranes were fixed in alcohol–formol (four parts of absolute alcohol and one part of 10% neutral formalin) and stained as previously described (8).

The following organs were also fixed in alcohol–formol, embedded in paraffin, cut, and stained with cresyl violet, or lightly with eosin alone (to better evaluate the carbon deposition): A) after the intra-carotid injections: the skin of the right cheek, the right

lip, the brain, the cerebellum, the right Gasserian ganglion, and the hypophysis; B) after intratesticular injections: the left and right epididymis, testes, cremaster muscles, and peritesticular fat.

Results. As the tables show, the intra-arterial injections of NaCl alone did not produce labeling of vessels in the treated territories. There were two minor exceptions, *i.e.*, scattered labeling of venules in the lip of one rat injected in the carotid, and in the peritesticular fat of another rat that had received the solution in the spermatic artery. However, even in these instances, the distribution and intensity of the labeling were not comparable to those observed after the administration of mediators. We attributed these exceptions to liberation of endogenous vasoactive substances due to the trauma of the experimental procedures. Irrespective of the treatment, colloidal carbon was always found in the lumen of the cavernous sinuses of the vibrissae. One insignificant carbon plug was found within the lumen of a small cerebral vessel (it was probably an embolus).

On the other hand, the administration of mediators in either the carotid or the spermatic arteries regularly induced carbon deposits in the wall of small vessels of the pertinent territories; the tissue spreads showed that the blood-borne particles were generally located in the wall of small venules (Fig. 1) (Tables I and II), occasionally extending to the wall of larger veins, but almost never in capillaries. However, not all the organs supplied by the injected arteries showed carbon deposits: none at all were found in the vessels of the brain, cerebellum, Gasserian ganglion, and testis (Fig. 2).

In the second set of experiments, the testicular vessels remained completely free of carbon deposits, whether the substance injected was histamine or NaCl, and whether the albuginea was intact or slit. At the site of the slit, some carbon was always present, mostly extravascular, some as intravascular plugs; this was clearly due to the direct vascular injury (1) caused by the incision. In the same animals, the subcutaneous injections serving as controls gave the expected results: histamine produced an area of vascular labeling; NaCl remained without effect.

Discussion. The principal differences between our experimental approach and that of previous studies of histamine-type mediators are: (1) that the agent reached the vessels from the endothelial side, and (2) that the agent reached the arteries before the venules. Despite these differences, the vascular segments of the microcirculation—in susceptible organs—responded as if the mediator had been injected into the tissue spaces: that is the labeling remained confined to the venules. We conclude that the selective effect of histamine-type mediators on the venules of susceptible organs is dependent upon an intrinsic property of the vascular tree and not on the route by which the mediator reaches the vessel.

It is remarkable that no trace at all of labeling appeared in the arterioles. This could not be ascribed to a more rapid passage of the drug through these vessels, because the duration of the injection should have allowed ample time for a histamine-type reaction to occur. Yet arteriolar leakage is known to occur under other conditions. Giese has described vascular labeling in arterioles (and "other small vessels") in the rat, after intravenous infusion of angiotensin, allegedly

TABLE II. Vascular Labeling After Injection of Histamine in the Internal Spermatic Artery.

		Vascular labeling									
		Meso of Epididymis		Epididymis		Testis		Cremaster		Peritesticular fat	
Gr	Treatment	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
1	NaCl	0	0	0	0	0	0	0	0	0	0
2	Histamine	+	0	+	0	0	0	+	0	+	0

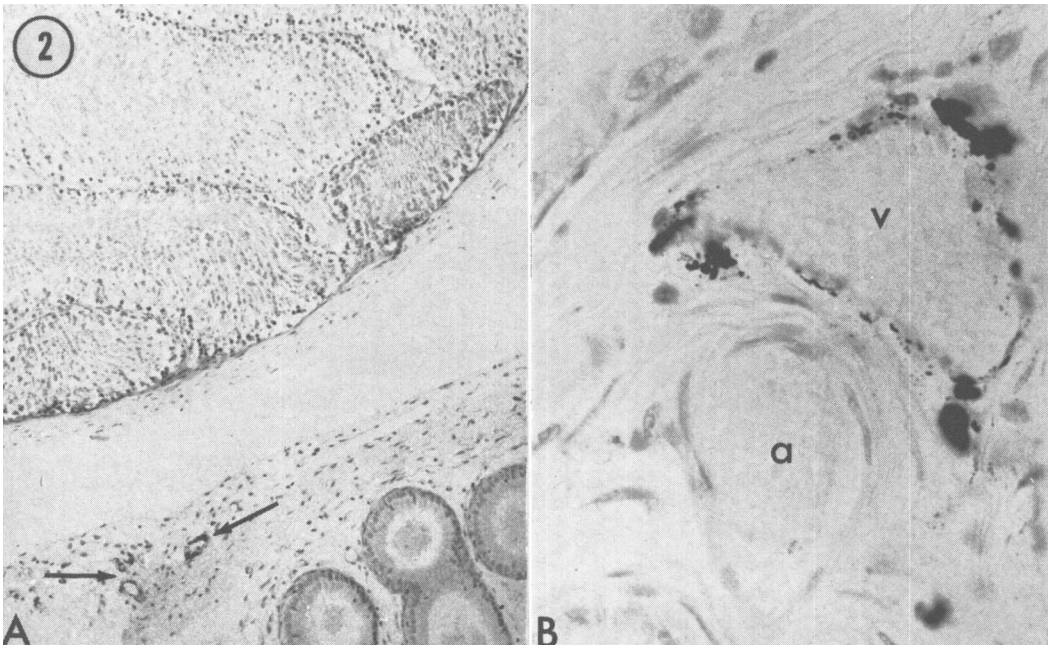

FIG. 2. Carbon deposition after intraspermatic artery injections of histamine. A. The carbon is present in the vessels of the epididymis (arrows) but not in those of the testis (×100). B. At higher magnification the carbon appears in the wall of a venule in the epididymis (×400).

as a mechanical result of hypertension (11). Though the mechanism is not clear, these experiments do show that arterioles can be induced to leak by pharmacological means, even though they do not leak after intra-arterial injection of histamine-type mediators. Focal arteriolar leakage has also been observed in experimental serum sickness; these lesions have been attributed to a local liberation of histamine-type mediators by clumps of platelets (12).

Another aspect of the selective action of histamine-type mediators is summarized in Tables I and II and in the results of the second series of experiments: that is their incapacity to cause vascular leakage in the small vessels of brain, cerebellum, Gasserian ganglion, and testis. It has been previously shown that small retinal vessels do not show increased carbon labeling after topical injection of histamine (16). It could be suspected that the amount of vasoactive compound injected plays a role in these differences; however, in experiments not reported here, we have tested a wide range of doses of mediators and always obtained results essentially similar to those described above. Moreover, the additional experiment based upon local administration of histamine in the testis showed that the compound fails to induce vascular labeling at its site of injection. Thus, one is led to conclude that in these organs the endothelium of the small vessels is functionally different from that of the venules of the skin and muscle. Pertinent ultrastructural peculiarities of these vessels have not yet been found; however, there are other reasons to believe that all the organs mentioned do have highly specialized endothelium: in the brain, the vessels differ with regard to their permeability, expressed by the blood–brain barrier (13); those of the Gasserian ganglion and testis are morphologically peculiar (in the rat) because of the high number of microvilli (9); a "barrier" has been described also in the testis in view of the behavior of its vessels after the injection of dyes, metals, and proteins (14, 15). There is also a clear-cut correlation between vascular response to mediators and distribution of

mast cells: at least two of the territories that did not show any vascular labeling after histamine (intravascular or topical) are known to have very few mast cells, if any: brain and testis (10). This is one of the few bits of evidence suggesting that the mast cells may have a local regulating function with regard to the microcirculation: a function possibly broader than that of increasing permeability, because in those tissues in which venules did respond to histamine, the distribution of the mast cells bore no consistent relationship to the venules. This was particularly evident in mounted preparations (8) such as periosteum of the calvarium and dura mater (Fig. 1).

In view of recent work, indicating that histamine-type mediators increase vascular permeability by causing endothelial cells to contract (7) and in view of the selective effect of these substances among different organs as well as among various segments of the same vascular tree, it seems most likely that the susceptible vessels contain endothelial cells endowed with a contractile system that is either absent or less effective in the others. Studies are under way to explore the possible ultrastructural basis of this difference.

Summary. Histamine, serotonin, and bradykinin, injected intra-arterially, cause (in susceptible organs) vascular leakage predominantly from the venules. The vascular segments affected are the same as when the mediators are injected locally. This suggests that the selective response of the venules depends on an intrinsic property of the venular wall, and not on the route whereby the mediator reaches the vessel. In certain organs (brain, cerebellum, testis, Gasserian ganglion) histamine-type mediators are apparently unable to cause vascular leakage.

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